Role of the NLRP3 inflammasome in stress-induced neuroinflammation and anxiety-like behavior

Undergraduate Honors Research Thesis

Presented in Partial Fulfillment of the Requirements for graduation
"with Honors Research Distinction in Neuroscience" in the undergraduate colleges of
The Ohio State University

by

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October 2017

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Introduction:

Exposure to chronic stress or traumatic events increases the risk of developing mood or anxiety disorders (1). In conjunction, mounting evidence indicates that heightened neuroinflammation is linked with the etiology of psychiatric disorders, including depression and anxiety (2). This inflammatory response is characteristic of the brain-to-immune and immune-to-brain communication essential for the development of mood and behavior. Previous studies have found that these specific brain regions are activated by stress. These are the pre-frontal cortex, hypothalamus, amygdala, and the CA3 and dentate gyrus areas of the hippocampus (3). These brain regions are critical for the induction of anxiety-like behavior due to stress exposure.

Stress changes the morphology of microglia within the brain activating them to foster an inflammatory response (4, 5). In support of the connection between microglia and behavioral changes, when microglia activation is blocked using minocycline, anxiety-like behavior following restraint stress is attenuated (6). Specifically, microglia release pro-inflammatory cytokines, such as IL-1β, which mediate changes to mood, cognition, and behavior that closely resemble characteristics observed in mood and anxiety disorders (7). Therefore, stress may increase vulnerability towards psychiatric disorders by enhancing neuroinflammatory processes.

Enhanced neuroinflammation is dependent on the upregulation of monocyte production in the bone marrow and trafficking to the blood brain barrier (8). $LYC6^{hi}$ monocytes in circulation traffic to the brain modulating the behavioral effects of chronic stress (9, 10). Blocking trafficking of monocytes during stress attenuates IL-1β

expression and prevents anxiety-like behavior (8, 11-13). Our lab's previous work provides novel evidence that anxiety-like behavior following stress is a result of microglial communication to peripheral monocytes, specifically those able to stimulate endothelial IL-1R1 by way of IL-1β (14).

In support of this, our laboratory uses the repeated social defeat (RSD) murine stress model to measure the effects of stress on neuroinflammation and behavior. RSD activates the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis (15). The effect of this activation is widely seen throughout myeloid cell population changes in the bone marrow, spleen, circulating blood, and the brain. Specifically, increased production of monocytes in the bone marrow, storage in the spleen, and increased trafficking to the brain is associated with the neuroinflammatory stress response. This has been confirmed using propranolol to block monocyte circulation and accumulation, resulting in decreased pro-inflammatory cytokine production (8, 16). Exposure to RSD stimulates monocyte trafficking from the bone marrow, specifically to fear and threat appraisal centers of the brain (17). This finding is significant because the monocyte population that traffics to the brain highly expresses IL-1β and propagates the neuroinflammation associated with the development of anxiety-like behavior (14). We hypothesize that IL-1β is a key signaling molecule for the neuroinflammatory and behavioral effects of stress exposure. However, the role of IL-1β in these processes is unknown.

IL-1β is a pro-inflammatory cytokine that is expressed by activated macrophages and microglia, and it causes inflammation in the CNS that promotes anxiety/ depressive like behaviors observed after stress (RSD) (11). IL-1β is tightly regulated and is

processed differently than other cytokines. IL-1β is transcribed into the precursor protein pro-IL-1β and requires enzymatic cleavage by caspase-1 to form mature IL-1β (18). Similarly, caspase-1 is activated following cleavage from pro-caspase-1 by the NLRP3 inflammasome. Thus, the NLRP3 inflammasome is a critical component for IL-1β to be released. Therefore, genetic knockout of NLRP3 is expected to prevent the release of IL-1β, which we hypothesize will inhibit depressive/anxiety-like behavior in response to RSD. Here we show that the peripheral immune response to stress is preserved when NLRP3 is genetically knocked out. This is confirmed by the increase of myeloid cell production in the bone marrow and increased myeloid cell populations in the spleen and in circulation. We also show that the NLRP3 inflammasome is not necessary for development of anxiety-like behavior in response to stress.

Methods:

Mice: Male C57BL/6 (6-8 weeks old) and male CD-1 (12 months old, retired breeders) mice were purchased from Charles River Breeding Laboratories (Wilmington, MA), and allowed to acclimate to their surroundings for 7-10 days prior to experiments. Transgenic NLRP3$^{-/-}$ mice were purchased from Jackson Labs and the colony was maintained at The Ohio State University. Resident C57BL/6 mice were housed in cohorts of three and aggressor CD-1 mice were individually housed. All mice were housed in 11.5”x 7.5”x 6” polypropylene cages. Rooms were maintained at 21°C under a 12-h light-dark cycle with *ad libitum* access to water and rodent chow. All procedures were in accordance with the National Institutes of Health Guidelines and were approved by The Ohio State University Institutional Animal Care and Use Committee.

Repeated Social Defeat (RSD): Mice were subjected to social stress (RSD). An aggressive male intruder CD-1 mouse was introduced into cages of established male cohorts (3 per cage) of C57BL/6 mice for 2 hours between 17:00 and 19:00 for six consecutive nights. During each cycle, submissive behavior (e.g., upright posture, fleeing, and crouching) was observed to ensure defeat of the resident mice. A new intruder was introduced if he did not initiate an attack on the resident mice within the first 5-10 minutes or if he was defeated by any of the resident mice (19). At the end of the 2 h period, the intruder was removed and the residents were left undisturbed until the following day when the paradigm was repeated. To avoid habituation, different intruders were used on consecutive nights. The health status of the mice was carefully examined throughout all experiments. Mice that were injured or moribund were removed from the study. Consistent with previous studies using RSD (20-23), less than 5% of mice met the early removal criteria. Control mice (CON) were left undisturbed in their home cages. All behavior and biological measures were obtained 14 h after the final cycle. This time point was selected because sympathetic nervous system and hypothalamic-pituitary-adrenal axis activation returns to baseline by 14 hours after the final cycle (16).

Isolation of Cells from Bone Marrow, Spleen, and Blood: Tissues were collected immediately following CO_2 asphyxiation. Whole blood was collected with EDTA-lined syringes by cardiac puncture and red blood cells were lysed. Spleens were collected in ice-cold phosphate buffered saline (PBS), mechanically disrupted to obtain single cell suspensions, and filtered through a 70-μm nylon cell strainer. Bone marrow was collected from the femur and flushed out with ice-cold PBS, then filtered through a 70-

μm nylon cell strainer. The total number of cells was determined with flow cytometry (19).

Isolation of Brain CD11b$^+$ Cells: CD11b$^+$ cells were isolated from whole-brain homogenates (17). In brief, brains were passed through a 70-μm nylon cell strainer and centrifuged at 900 x *g* for 6 min. Supernatants were removed and cell pellets were re-suspended in 70% isotonic Percoll (GE-Healthcare, Chicago, Illinois). A discontinuous Percoll density gradient was layered as follows: 50%, 35%, and 0% isotonic Percoll. The gradient was centrifuged for 20 min at 2000 x *g* and cells were collected from the interphase between the 70% and 50% Percoll layers. These cells were referred to as enriched brain CD11b$^+$ cells based on previous studies demonstrating that viable cells isolated by Percoll density gradient yields >90% CD11b$^+$ cells (17).

Flow Cytometry: FC receptors were blocked with anti-CD16/CD32 antibody (eBioscience; catalog number 553142). Cells were incubated with the appropriate antibodies (CD45, CD11b, CD115, eBioscience; Ly6C, BD Biosciences, Franklin Lakes, NJ) for 1 h at 4°C. Cells were washed, pelleted, and then re-suspended in PBS for analysis. Cell numbers were estimated using counting beads (BD Biosciences). Non-specific binding was assessed using isotype-matched antibodies. Antigen expression was determined using a Becton-Dickinson FACSCalibur four-color cytometer (BD Biosciences). Data were analyzed using FlowJo software (Tree Star, Ashland, OR) and positive labeling for each antibody was determined based on isotype stained controls.

RNA Isolation and Real-Time PCR: A 1 mm coronal brain section that included the cortex, hippocampus, striatum, and hypothalamus was removed and immediately flash

frozen in liquid nitrogen. RNA was isolated using tri-reagent/isopropanol precipitation and RNA concentration was determined by NanoPhotometry (Implen, Munich, Germany). RNA (1.2μg) was reverse transcribed to cDNA using an RT-RETROscript kit (Ambion, ThermoFisher, Waltham, MA). For Percoll-enriched microglia, the PrepEase kit (USB, CA) was used to isolate RNA according to the manufacturer's instructions. Real-time quantitative PCR was performed using the Applied Biosystems Assay-on-Demand Gene Expression protocol. Experimental cDNA was amplified by real-time PCR where a target cDNA and reference cDNA (glceraldehyde-3-phosphate dehydrogenase (GAPDH)) were amplified simultaneously using an oligonucleotide probe with a 5' fluorescent reporter dye (FAM) and a 3' quencher dye (non-fluorescent quencher). Florescence was determined on an ABI PRISM 7300 sequence detection system (Applied Biosystems). Relative gene expression was analyzed using the ΔΔCT method (24) and results are expressed as fold difference from GAPDH.

Social Interaction: Social avoidance behavior was determined by measuring interaction time of subject mouse with an unfamiliar CD-1 mouse. The experimental mouse was placed in the arena containing an unfamiliar CD-1 mouse in a wire mesh cage. Activity was recorded for 5 min. Activity in the social avoidance behavior test was recorded and analyzed using Noldus EthoVision XT Software (19).

Anxiety-Like Behavior: Anxiety-like behavior was determined using open-field activity. The open-field test was used to assess anxiety-like behavior in these studies because our previous work shows that it is a robust and reproducible behavioral test in the context of social defeat. Furthermore, measures of thigmotaxis in the open-field test demonstrate high degrees of validity for modeling anxiety-like behaviors. For the open-

field test, mice were placed in the corner of the test apparatus (40 × 40 × 25 cm Plexiglas box) and activity was recorded for 5 min. Mice with anxiety-like behavior enter the center of the open-field slower and spend less time in the open-field (19). Behavior was recorded and analyzed using an automated system (VersaMax, AccuScan Instruments, Omnitech Electronics Inc., Columbus, OH).

Statistical Analysis: All data are expressed as treatment means ± standard error of the mean (SEM). Individual data points more than two standard deviations above and below the mean were counted as outliers, and were excluded in the subsequent analyses. To determine significant main effects and interactions between main factors, data were analyzed using one-way or two-way ANOVA using the General Linear Model procedures of SPSS statistical software (IBM, Armonk, NY). In the event of a main effect of experimental treatment, differences between group-means were evaluated by an F-protected t-test. *Post hoc* analyses are graphically presented in figures. Threshold for statistical significance was set at $p < 0.05$.

Results:

RSD increases myelopoiesis in the bone marrow independent of NLRP3. The bone marrow is a primary site of hematopoiesis. Our laboratory has previously shown that the development of prolonged anxiety-like behavior during RSD is mediated by enhanced proliferation of inflammatory monocytes. These monocytes traffic from the bone marrow to fear and threat appraisal centers of the brain (14). Thus, the first objective was to determine whether the NLRP3 inflammasome regulated myelopoiesis following exposure to RSD. Overall, stress significantly increased the amount of granulocytes

(main effect of stress; $F_{1,42}$= 220.4; p<0.05) and LYC6^{hi} monocytes (main effect of stress; $F_{1,40}$=72.8; p<0.05) (Fig.1a,1b) in the bone marrow. In addition, stress significantly decreased the amount of lymphocytes (main effect of stress; $F_{1,41}$= 643.3; p<0.05) and RBCs (main interaction between genotype and stress; $F_{1,42}$= 9.05; p<0.05) (Fig.1c,1d). These results demonstrate that the NLRP3 inflammasome has no effect on myelopoiesis within the bone marrow after stress.

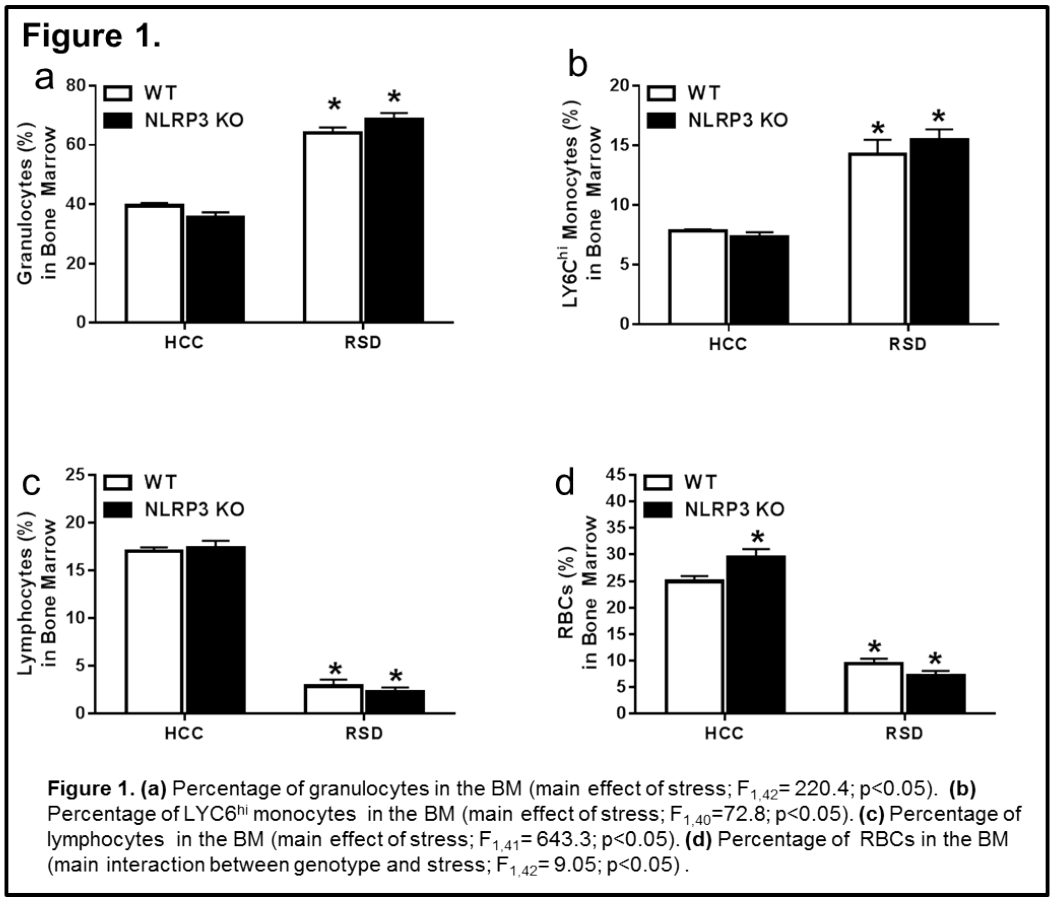

Figure 1. (a) Percentage of granulocytes in the BM (main effect of stress; $F_{1,42}$= 220.4; p<0.05). **(b)** Percentage of LYC6^{hi} monocytes in the BM (main effect of stress; $F_{1,40}$=72.8; p<0.05). **(c)** Percentage of lymphocytes in the BM (main effect of stress; $F_{1,41}$= 643.3; p<0.05). **(d)** Percentage of RBCs in the BM (main interaction between genotype and stress; $F_{1,42}$= 9.05; p<0.05) .

RSD increases circulating granulocytes and monocytes independent of NLRP3. As described in previous studies, myelopoiesis in the bone marrow causes increased accumulation of Ly6C^{hi} monocytes in circulation. Considering myelopoiesis from the bone marrow was not attenuated in NLRP3 KO mice exposed to RSD, the NLRP3

inflammasome was not expected to be involved in the release of myeloid cells from the BM into circulation. Here, we report that the genetic knockout of NLRP3 does not alter the increase in granulocytes in circulation in response to stress (main effect of stress; $F_{1,41}$= 81.2; p<0.05) (Fig.2a). Post-hoc analysis revealed that RSD increased granulocytes in the blood in WT (p<0.05; HCC:WT x RSD:WT) and NLRP3 KO (p<0.05; HCC:WT x RSD:NLRP3) mice. Additionally, exposure to RSD increased circulating monocytes, independent of genotype. (main effect of stress; $F_{1,39}$= 46.94; p<0.05) (Fig.2b). Post-hoc analysis revealed that RSD increased monocytes in the blood in WT mice (p<0.05; HCC:WT x RSD:WT) and NLRP3 KO (p<0.05; HCC:WT x RSD:NLRP3 KO) mice. These results demonstrate that the NLRP3 inflammasome was not involved in the elevation of circulating myeloid cells following stress.

Figure 2.

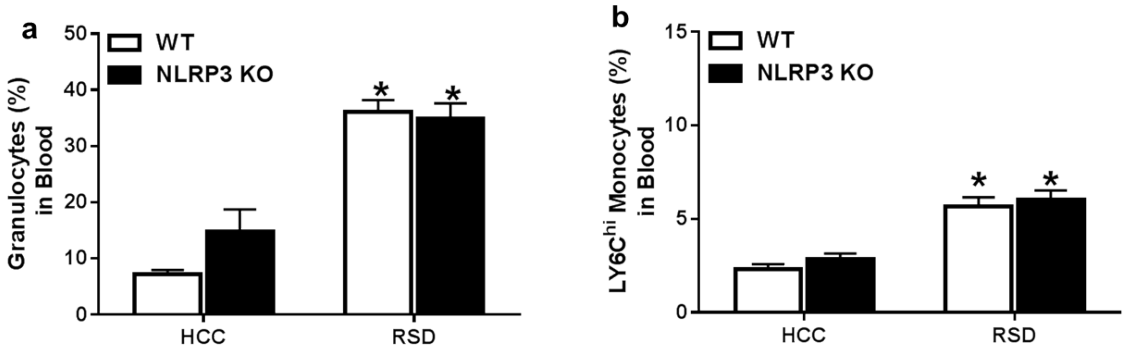

Figure 2. (a) Percentage of granulocytes in the blood (main effect of stress; $F_{1,41}$= 81.2; p<0.05) **(b)** Percentage of LYC6^{hi} monocytes in the blood (main effect of stress; $F_{1,39}$= 46.94; p<0.05).

RSD increases splenic myeloid cells independent of NLRP3. The spleen acts as an important reservoir of monocytes during inflammatory conditions that maintain stress-sensitization to subsequent stressors. We have previously reported that mice exposed to RSD had increased numbers of monocytes and granulocytes in the spleen (25). The

goal of this experiment was to determine whether the NLRP3 inflammasome mediates myeloid cell redistribution following RSD. Stress significantly increased granulocytes in the spleen, which was attenuated by genetic knockout of NLRP3 (main interaction between stress and genotype;$F_{1,37}$=6.72; p<0.05) (Fig.3a). Post-hoc analysis revealed that RSD increased granulocytes in the spleen in WT (p<0.05; HCC:WT x RSD:WT) and NLRP3 KO (p<0.05; HCC:WT x RSD:NLRP3) mice. However, genetic KO of NLRP3 significantly reduced the granulocyte population in RSD exposed mice (p<0.05; RSD:WT x RSD:NLRP3 KO). In addition, genetic deletion of NLRP3 did not alter the monocyte population in the spleen (main effect of stress; ($F_{1,37}$=97.53; p<0.05) (Fig.3b). Post-hoc analysis revealed that exposure to RSD increased LY6C^{hi} Monocytes in the spleen in WT (p<0.05; HCC:WT x RSD:WT) and NLRP3 KO (p<0.05; HCC:WT x RSD:NLRP3) mice. Taken together, the NLRP3 inflammasome reduced granulocytes in the spleen and the brain but had no effect on monocyte redistribution following RSD.

Figure 3.

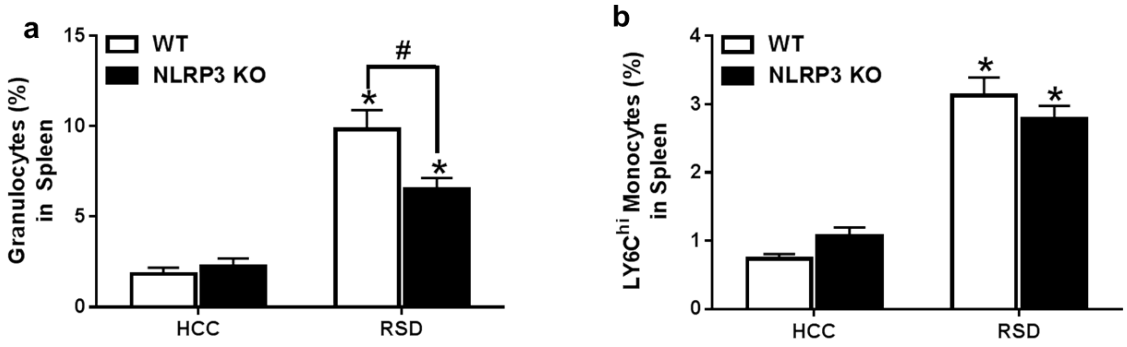

Figure 3. (a) Percentage of granulocytes in the spleen (main interaction between stress and genotype; $F_{1,37}$=6.72; p<0.05) . **(b)** Percentage of LYC6^{hi} monocytes in the spleen (main effect of stress; $F_{1,37}$=97.53; p<0.05) .

RSD promotes trafficking of granulocytes and monocytes to the brain independent of the NLRP3 inflammasome. RSD has previously been shown to increase trafficking of myeloid cells from the BM to the brain (17). Therefore, we wanted

to determine whether the NLRP3 inflammasome was necessary for myeloid cell trafficking to the brain. Here we report that the lymphocyte population in the brain was not significantly altered by stress or genotype (Fig.4a). Exposure to RSD increased trafficking of granulocytes to the brain (main effect of stress; $F_{1,37}$; 14.86; $p<0.05$) (Fig.4b). Post-hoc analysis revealed RSD increased granulocyte population in the brain for WT mice ($p<0.05$; HCC:WT x RSD:WT) and this increase was attenuated in NLRP3 KO mice ($p<0.05$; RSD:WT x RSD:NLRP3 KO). RSD also increased monocytes in the brain independent of genotype (main effect of stress; $F_{1,39}= 32.97$; $p<0.05$) (Fig.4c). Post-hoc analysis revealed RSD increased monocytes in the brain in both WT ($p<0.05$; HCC:WT x RSD:WT) and NLRP3 KO groups ($p<0.05$; HCC:NLRP3 KO x RSD: NLRP3 KO). These results show trafficking of granulocytes and monocytes to the brain is independent from the NLRP3 inflammasome.

Figure 4.

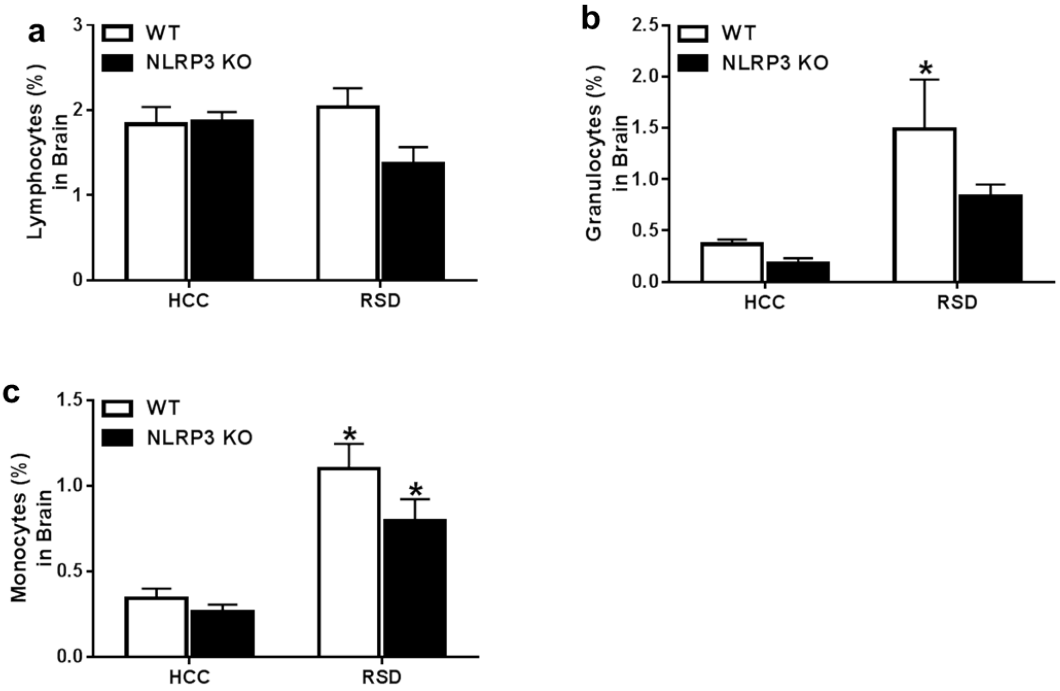

Figure 4. (a) Percentage of lymphocytes in the brain. **(b)** Percentage of granulocytes in the brain (main effect of stress; $F_{1,37}$; 14.86; $p<0.05$). **(c)** Percentage of monocytes in the brain (main effect of stress; $F_{1,39}$= 32.97; $p<0.05$) .

Effect of NLRP3 on RSD-induced neuroinflammatory signaling IL-1β, IL-6, and TNFα. Mounting evidence implicates elevated neuroinflammation in the etiology of mood disorders (26). We previously reported increased expression of IL-1β following exposure to RSD (25). Here, we report that genetic knockout of the NLRP3 inflammasome did not disrupt stress-induced IL-1β mRNA expression (main effect of stress; $F_{1,40}$=7.658; $p<0.05$) (Fig.5a). There was a main interaction of IL-6 mRNA between genotype and stress ($F_{1,41}$=5.617; $p<0.05$) (Fig.5b). Post-hoc analysis revealed that IL-6 mRNA in NLRP3 KO mice were significantly increased in HCC mice ($p<0.05$; HCC:WT x HCC:NLRP3 KO). Additionally, exposure to RSD decreased IL-6 mRNA in

NLRP3 KO mice ($p<0.05$; HCC:NLRP3 KO x RSD:NLRP3 KO). Gene expression for TNFα was increased in NLRP3 KO mice (main effect of genotype; $F_{1,41}=6.021$; $p<0.05$) (Fig.5c). Post-hoc analysis revealed the NLRP3 KO group exposed to RSD had increased TNFα expression ($p<0.05$; HCC:WT x RSD:NLRP3 KO). Overall, genetic knockout of NLRP3 did not interfere with IL-1β mRNA expression following stress.

Figure 5.

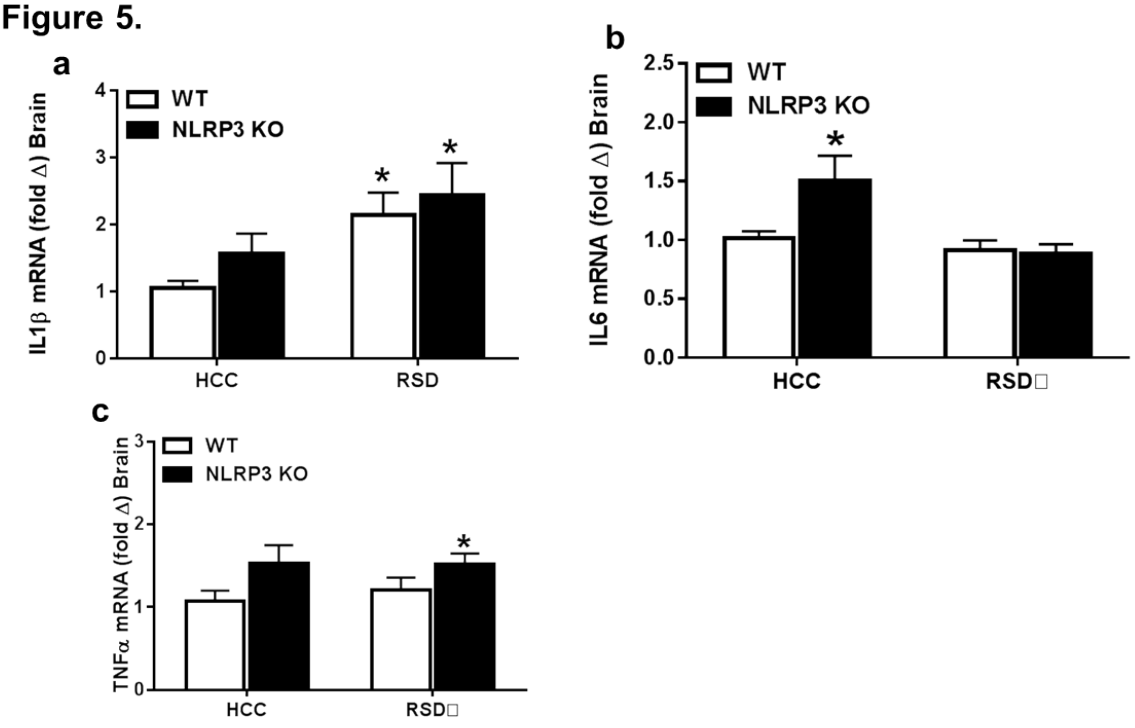

Figure 5. (a) IL-1β mRNA levels in the brain (main effect of stress; $F_{1,40}=7.658$; $p<0.05$) . **(b)** IL-6 mRNA levels in the brain (main interaction; $F_{1,41}=5.617$; $p<0.05$). **(c)** TNFα mRNA levels in the brain (main effect of genotype; $F_{1,41}=6.021$; $p<0.05$) .

Effect of NLRP3 on anxiety-like behavior. RSD promotes anxiety-like and depressive-like behavior (25). We aimed to determine whether anxiety-like behavior and/or social avoidance would be affected by genetic knockout of NLRP3. Social avoidance was assessed by measuring sociability to an unfamiliar CD-1 mouse.

Overall, mice exposed to RSD, regardless of genotype, spent more time in the corner zones (main effect of stress; $F_{1,59}$= 13.99; $p<0.05$) (Fig.6a). Post-hoc analysis revealed a significant increase in time spent in corner in response to RSD in NLRP3 KO mice ($p<0.05$; HCC:NLRP3 KO x RSD:NLRP3 KO). Post-hoc analysis also revealed a significant difference between genetic KO of NLRP3 in RSD groups and WT groups ($p<0.05$; RSD:WT x RSD:NLRP3 KO). In line with these results, RSD exposed mice spent less time in the interaction zone, regardless of genotype (main effect of stress; $F_{1,61}$= 0.003; $p<0.05$) (Fig.6b). Post-hoc analysis revealed significant decrease in time spent interacting in response to RSD in NLRP3 KO mice ($p<0.05$; HCC:NLRP3 KO x RSD:NLRP3 KO). Post-hoc analysis also revealed a significant difference between genetic KO of NLRP3 in RSD groups ($p<0.05$; RSD:WT x RSD:NLRP3 KO). These findings show the NLRP3 inflammasome did not mediate the social avoidant behavior displayed following stress.

Similarly, the open-field paradigm has been used in our lab previously to measure anxiety-like behavior following stress (20, 27). We aimed to examine the necessity of the NLRP3 inflammasome in the promotion of anxiety-like behavior following stress. Interestingly, there was no difference in time spent in the center of the field or latency to enter the center across all groups (Fig.6c,6d). The genetic KO of NLRP3 did not significantly alter time spent in the center or latency to enter the center of the open field arena. Overall, behavioral testing unexpectedly showed the NLRP3 inflammasome does not play a critical role in inducing anxiety-like behavior following stress.

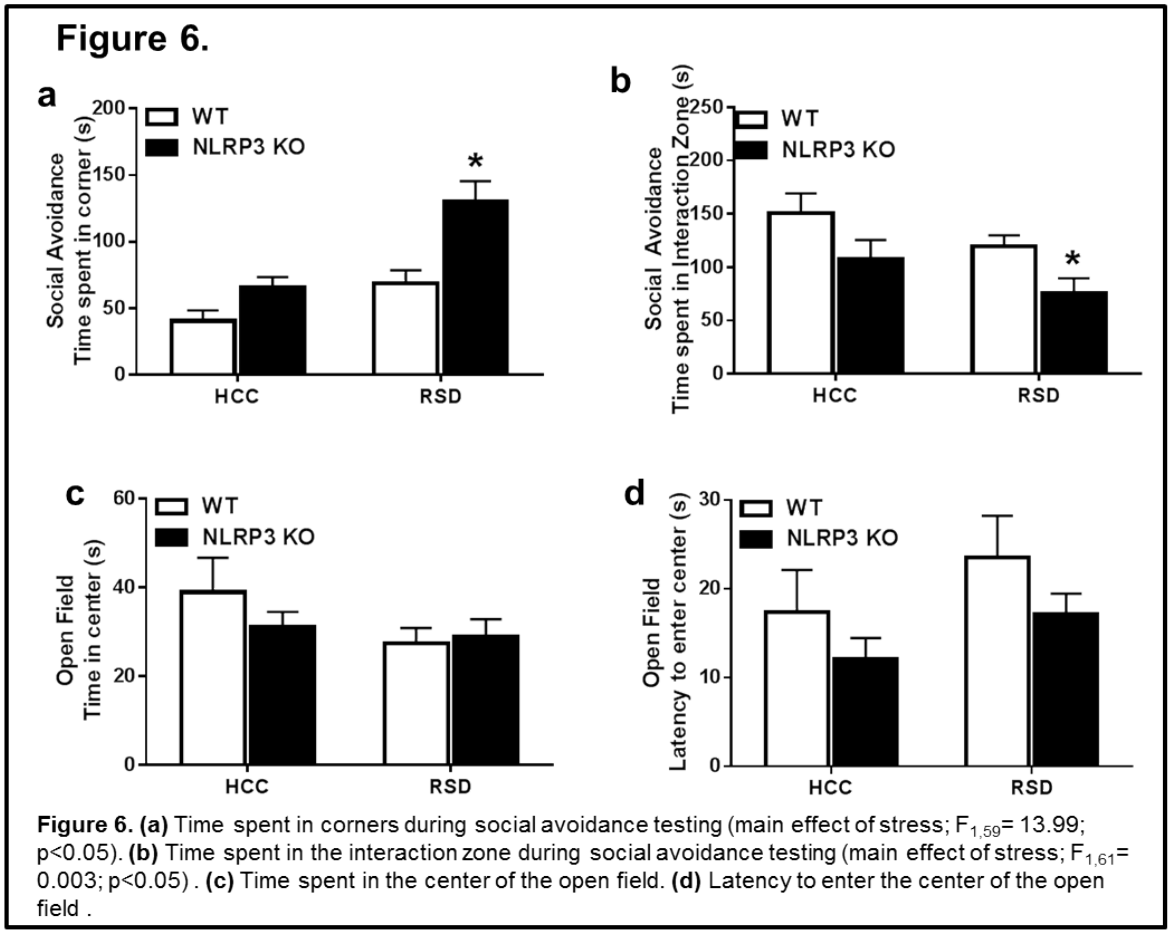

Figure 6. (a) Time spent in corners during social avoidance testing (main effect of stress; $F_{1,59}$= 13.99; p<0.05). **(b)** Time spent in the interaction zone during social avoidance testing (main effect of stress; $F_{1,61}$= 0.003; p<0.05) . **(c)** Time spent in the center of the open field. **(d)** Latency to enter the center of the open field .

Discussion:

Chronic stress is a predisposing factor for mood disorders which affect a growing number of people (28, 29). Stress-induced mood disorders have fostered increased interest in neuroinflammation as the mediator of these physiological and behavioral outputs. The aim of the present study was to identify underlying mechanisms of neuroimmune signaling that mediates stress-induced behaviors. Previous work established IL-1β to be necessary for developing anxiety-like behavior in response to

stress (17). To further understand how IL-1β is activated, the role of NLRP3 in neuroinflammatory stress response was necessary. Our first key finding was that genetic KO of NLRP3 did not alter the peripheral immune response to RSD. Moreover, myeloid cell trafficking to the brain in response to stress was not significantly changed by the genetic KO of NLRP3. Additionally, stress-induced immune cell signaling via pro-inflammatory cytokines was preserved. Lastly, the development of anxiety-like behavior was found to be independent of the NLRP3 inflammasome.

The development of anxiety following RSD depends on increased production of inflammatory monocytes from the bone marrow that traffic to the brain (14). Our first main finding was that genetic knockout of the NLRP3 inflammasome did not prevent myelopoiesis in mice exposed to RSD. Stress-induced release of inflammatory monocytes occurs via activation of the sympathetic nervous system and release of norepinephrine into the bone marrow (30), (31) (32). For example, blocking the sympathetic nervous system response to stress via B-adrenergic receptor antagonism attenuated an enlarged spleen (i.e. splenomegaly), anxiety-like behavior, and IL-6 response to stress (16). Thus, the sympathetic nervous system and catecholamine release, not the NLRP3 inflammasome, are responsible for stress-induced myelopoiesis. In addition, mice treated with benzodiazepines and exposed to RSD presented with attenuated anxiety-like behavior. Myeloid cell trafficking to the brain through pro-inflammatory cytokine mediated signaling was prevented. Benzodiazepines were found to inhibit the HPA axis response to stress in addition to inhibiting anxiety-like behavior driven in part by corticosterone release (33). Considering benzodiazepine blocked the sympathetic nervous system and that KO of NLRP3 did not significantly

impact the peripheral immune response, we conclude that the activation of the sympathetic nervous system occurs independently of the NLRP3 inflammasome.

Exposure to RSD increases the proliferation of BM-derived monocytes and granulocytes, which traffic into circulation (34), (35). The increase of these cells in circulation suggests a probable signal from the nervous system to initiate release of these cells from the bone marrow. For example, microglia are thought to be responsible for the recruitment of IL-1β producing monocytes from the periphery (14). This communication is of interest considering the role of the NLRP3 inflammasome in cleaving pro-caspase-1 to caspase-1, which cleaves pro-IL-1β to active IL-1β. Considering the genetic KO of NLRP3 did not attenuate the increase of granulocytes or monocytes in circulation, the signaling process to increase quantity of peripheral immune cells is not mediated by the presence of NLRP3. This is supported by our previous finding that genetic deletion of caspase-1 in BM-derived cells did not prevent the accumulation of monocytes in circulation after exposure to RSD (14). These findings indicate that the accumulation of myeloid cells in circulation occurs independently of IL-1β.

Previous work indicated splenomegaly following exposure to RSD. The spleen acts as a reservoir of primed monocytes receiving neuroendocrine signals to be released and traffic to the brain (19). Our second major finding is NLRP3 does not mediate this increased splenic myeloid cell population. This is in line with established understanding of the splenic and overall peripheral immune profile after RSD. Splenectomy inhibiting monocyte trafficking and anxiety-like behavior following stress is

supporting evidence for it's critical role in the peripheral immune response to stress (20).

Our findings highlight the complex cellular interactions required to recruit myeloid cells to the brain. For example, genetic deletion of NLRP3 attenuated the trafficking of granulocytes to the brain following stress. Notably, IL-1β is a pro-inflammatory cytokine that stimulates granulocyte chemotactic factors such as CXCL8. Therefore, one possibility is that NLRP3 mediates granulocyte trafficking to the brain via IL-1β stimulation of IL-8. In addition, we report NLRP3 knockout did not prevent monocyte trafficking to the brain. This is supported by our previous finding that genetic deletion of caspase-1 in BM-derived cells did not prevent monocyte trafficking to the brain after exposure to RSD (14). These findings indicate that IL-1β differentially regulates stress-induced trafficking of granulates and monocytes to the brain.

Social avoidant behavior is driven through an established neuronal mechanism (27). NLRP3 was shown to not play a significant role in the social interaction of mice dependent on stress. Open-field behavior was inconclusive as to the role NLRP3 plays in facilitating anxiety-like behavior due to stress. We hypothesized that NLRP3 is necessary for occurrence of anxiety-like behavior. As previously established (25), exposure to stress causes IL-1β upregulation that is implicated in the development of anxiety-like behavior. Considering the NLRP3 inflammasome activates IL-1β, the attenuation of behavior was expected following genetic deletion of NLRP3. This suggests the potential upregulation of other inflammasome complexes which may facilitate IL-1B activity and preserve behavioral response to stress.

Conclusion:

The findings of this study are an important step to uncovering the role of neuroinflammation due to stress causing debilitating anxiety-like effects. Through the present findings, our lab will continue to explore the role of IL-1β and other pro-inflammatory cytokines as well as the mechanism of action for the inflammatory reaction to stress. NLRP3 was a critical target to address the role of IL-1β in immune and behavioral responses to stress. Overall, NLRP3 is not responsible for stress-induced peripheral inflammatory response and does not mediate the presentation of anxiety-like behavior. Future studies will add significance to the behavioral stress response and increase current understanding of the neuroinflammatory link to stress.

Acknowledgements:

This study was supported by National Institute of Health (NIMH) grants R01-MH-093473 and R01-MH093472 to JFS. DBM and MDW were supported by NIDCR Training Grant T32-DE014320. DBM was supported by F31-MH-109234. Thank you to the Institute of Behavioral Medicine Research students and employees; Anzela, Caroline, Damon, Kristina, Shane, Danny, Natalie, Dave, and Yufen. Thank you Dr. Mike Weber, Dr. Dan McKim, Dr. Sheridan, and Dr. Godbout for teaching me how to be a diligent scientist.

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